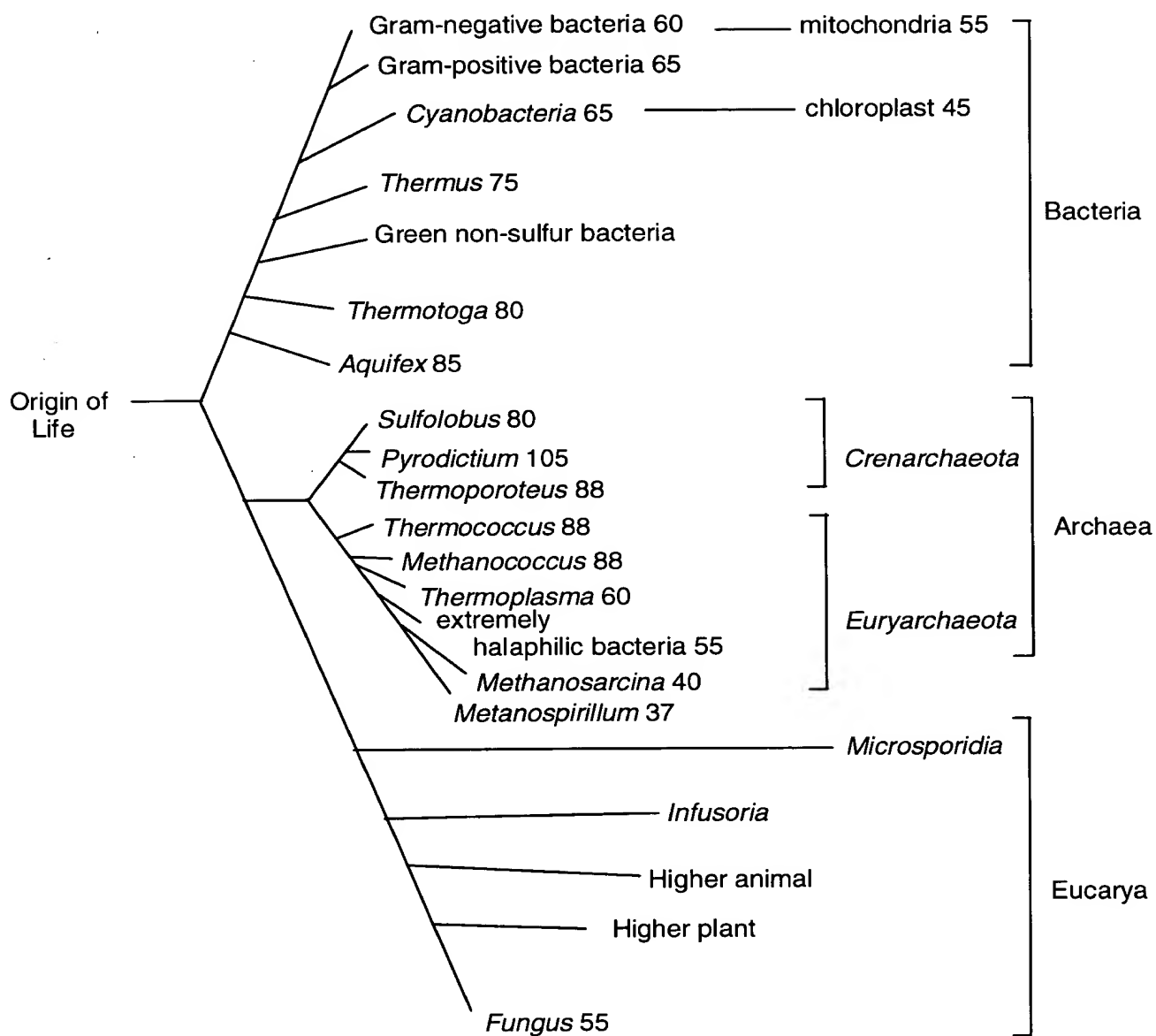


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SHEET 1 OF 13

1/13

FIG. 1



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SHEET 2 OF 13

2/13

FIG. 2

		89	97	149	157	256	263	280	285
IPMDH	<i>Sulfolobus sp. strain 7</i>	YDMYANIRP---	IAKVG-	LNFA---	VHGA	AFDI---	MMY	ERM	
	<i>Thermus thermophilus</i>	QDLFANLRP---	VARVA-	FEAA---	VHGS	APDI---	MMLE	HA	
	<i>Bacillus subtilis</i>	LDLFANLRP---	VIREG-	FKMA---	VHGS	APDI---	MLLR	TS	
	<i>Escherichia coli</i>	FKLFSNLRP---	IARIA-	FESA---	AGGS	APDI---	LLLR	YS	
	<i>Agrobacterium tumefaciens</i>	LELFANLRP---	IASVA-	FELA---	VHGS	APDI---	MCLR	YS	
	<i>Saccharomyces cerevisiae</i>	LQLYANLRP---	ITRMA	AF-MA---	CHGS	APDL---	MMLK	LS	
	<i>Neurospora crassa</i>	LGTYGNLRP---	IARLAG	F-LA---	IHGS	APDI---	MMLR	YS	
ICDH	<i>Saccharomyces cerevisiae</i>	FGLFANVRP---	VIRYA-	FEYA---	VHGS	APDI---	MMLN	NHM	
	<i>Bos taurus (3/4)</i>	FDLYANVRP---	IAEFA-	FEYA---	VHGT	APDI---	MMLR	RHM	
	<i>Bacillus subtilis</i>	LDL FVCLRP---	LVRAA-	IDYA---	THGT	APKY---	LLLE	HL	
	<i>Escherichia coli</i>	LDLYICLRP---	LVRAA-	IEYA---	THGT	APKY---	MMLR	RHM	
	<i>Ancestral residues</i>	xDLxANLRP---	IARxA	xFExA---	VHGS	APDI---	MMLx	xxx	

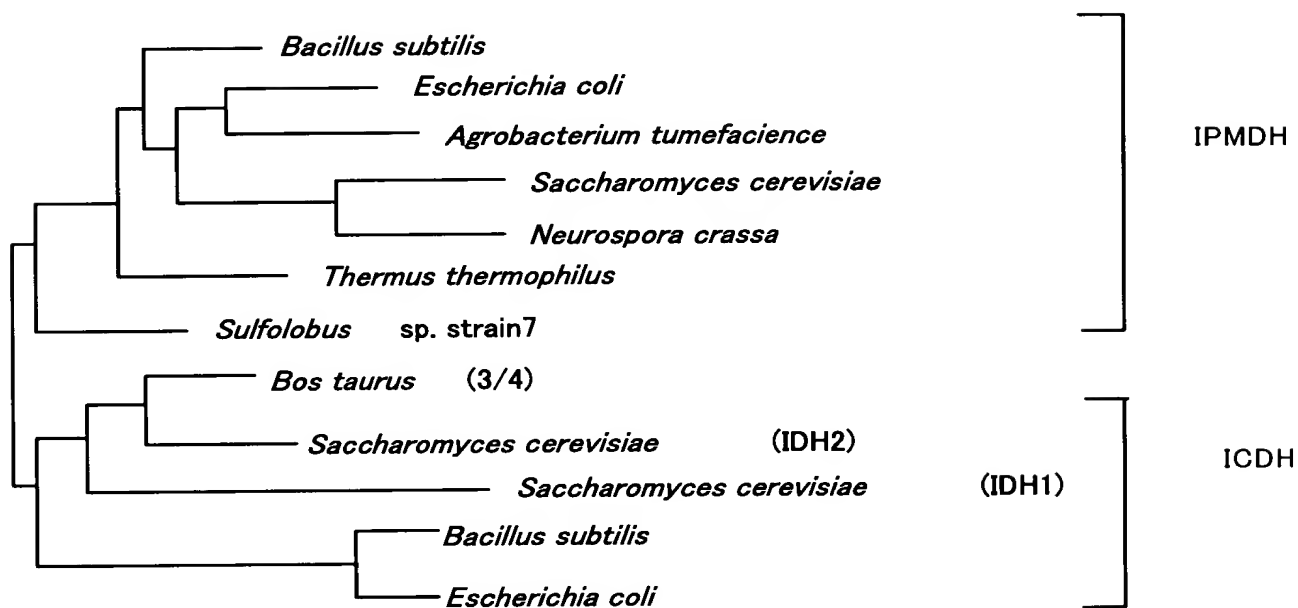
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SHEET 3 OF 13

3/13

FIG. 3



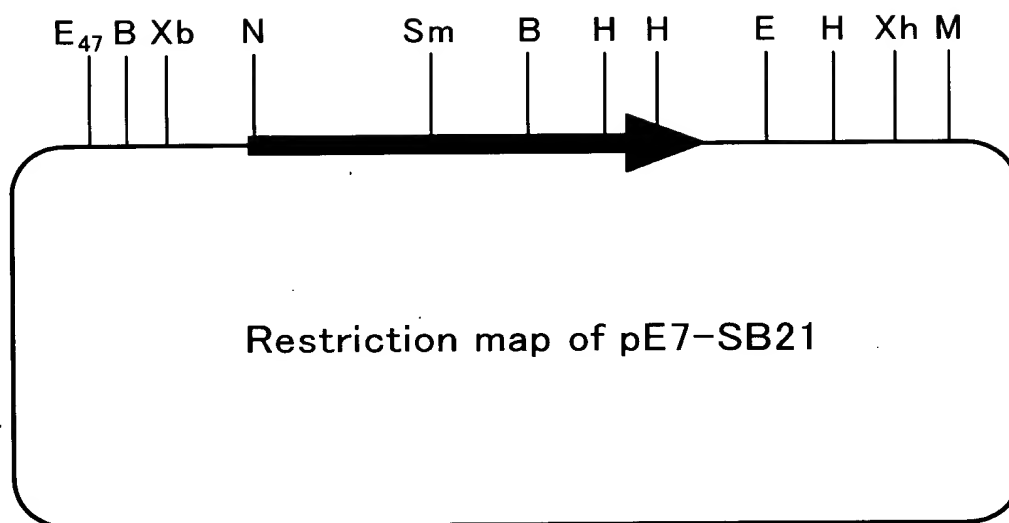
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SHEET 5 OF 13

5/13

FIG. 5



Restriction map of pE7-SB21

E₄₇: *Eco47* III, B: *Bgl* II, Xb: *Xba* I, N: *Nde* I, Sm: *Sma* I, H: *Hind* III, E: *EcoR* I, Xh: *Xho* I, M: *Mro* I

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SHEET 6 OF 13

6/13

FIG. 6

atg ggc ttt act gtt gct tta ata caa gga gat gga att gga cca gaa 48
Met Gly Phe Thr Val Ala Leu Ile Gln Gly Asp Gly Ile Gly Pro Glu 16

ata gta tct aaa tct aag aga ata tta gcc aaa ata aat gag ctt tat 96
Ile Val Ser Lys Ser Lys Arg Ile Leu Ala Lys Ile Asn Glu Leu Tyr 32

tct ttg cct atc gaa tat att gaa gta gaa gct ggt gat cgt gca ttg 144
Ser Leu Pro Ile Glu Tyr Ile Glu Val Glu Ala Gly Asp Arg Ala Leu 48

gca aga tat ggt gaa gca ttg cca aaa gat agc tta aaa atc att gat 192
Ala Arg Tyr Gly Glu Ala Leu Pro Lys Asp Ser Leu Lys Ile Ile Asp 64

aag gcc gat ata att ttg aaa ggt cca gta gga gaa tcc gct gca gac 240
Lys Ala Asp Ile Ile Leu Lys Gly Pro Val Gly Glu Ser Ala Ala Asp 80

Primer P1 annealing site

gtt gtt gtc aag tta aga caa att tat gat atg tat gcc aat att aga 288
Val Val Val Lys Leu Arg Gln Ile Tyr Asp Met Tyr Ala Asn Ile Arg 96

cca gca aag tct atc ccg gga ata gat act aaa tat ggt aat gtt gat 336
Pro Ala Lys Ser Ile Pro Gly Ile Asp Thr Lys Tyr Gly Asn Val Asp 112

ata ctt ata gtg aga gaa aat act gag gat tta tac aaa ggt ttt gaa 384
Ile Leu Ile Val Arg Glu Asn Thr Glu Asp Leu Tyr Lys Gly Phe Glu 128

cat att gtt tct gat gga gta gcc gtt ggc atg aaa atc ata act aga 432
His Ile Val Ser Asp Gly Val Ala Val Gly Met Lys Ile Ile Thr Arg 144

Primer P4 annealing site

ttt gct tct gag aga ata gca aaa gta ggg cta aac ttt gca tta aga 480
Phe Ala Ser Glu Arg Ile Ala Lys Val Gly Leu Asn Phe Ala Leu Arg 160

agg aga aag aaa gta act tgt gtt cat aag gct aac gta atg aga att 528
Arg Arg Lys Lys Val Thr Cys Val His Lys Ala Asn Val Met Arg Ile 176

act gat ggt tta ttc gct gaa gca tgc aga tct gta tta aaa gga aaa 576
Thr Asp Gly Leu Phe Ala Glu Ala Cys Arg Ser Val Leu Lys Gly Lys 192

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INV: Akihiko YAMAGISHI
SHEET 7 OF 13

7/13

FIG. 7

gta gaa tat tca gaa atg tat gta gac gca gca gcg gct aat tta gta 624
Val Glu Tyr Ser Glu Met Tyr Val Asp Ala Ala Ala Asn Leu Val 208

aga aat cct caa atg ttt gat gta att gta act gag aac gta tat gga 672
Arg Asn Pro Gln Met Phe Asp Val Ile Val Thr Glu Asn Val Tyr Gly 224

gac att tta agt gac gaa gct agt caa att gcg ggt agt tta ggt ata 720
Asp Ile Leu Ser Asp Glu Ala Ser Gln Ile Ala Gly Ser Leu Gly Ile 240

Primer P5

gca ccc tct gcg aat ata gga gat aaa aaa gct tta ttt gaa cca gta 768
Ala Pro Ser Ala Asn Ile Gly Asp Lys Lys Ala Leu Phe Glu Pro Val 256

annealing site

cac ggt gca gcg ttt gac att gct gga aag aat ata ggt aat ccc act 816
His Gly Ala Ala Phe Asp Ile Ala Gly Lys Asn Ile Gly Asn Pro Thr 272

Primer P6 annealing site

gca ttt tta ctt tct gta agt atg atg tat gaa aga atg tat gag cta 864
Ala Phe Leu Leu Ser Val Ser Met Met Tyr Glu Arg Met Tyr Glu Leu 288

tct aat gac gat aga tat ata aaa gct tca aga gct tta gaa aac gct 912
Ser Asn Asp Asp Arg Tyr Ile Lys Ala Ser Arg Ala Leu Glu Asn Ala 304

ata tac tta gtc tac aaa gag aga aaa gcg tta acc cca gat gta ggt 960
Ile Tyr Leu Val Tyr Lys Glu Arg Lys Ala Leu Thr Pro Asp Val Gly 320

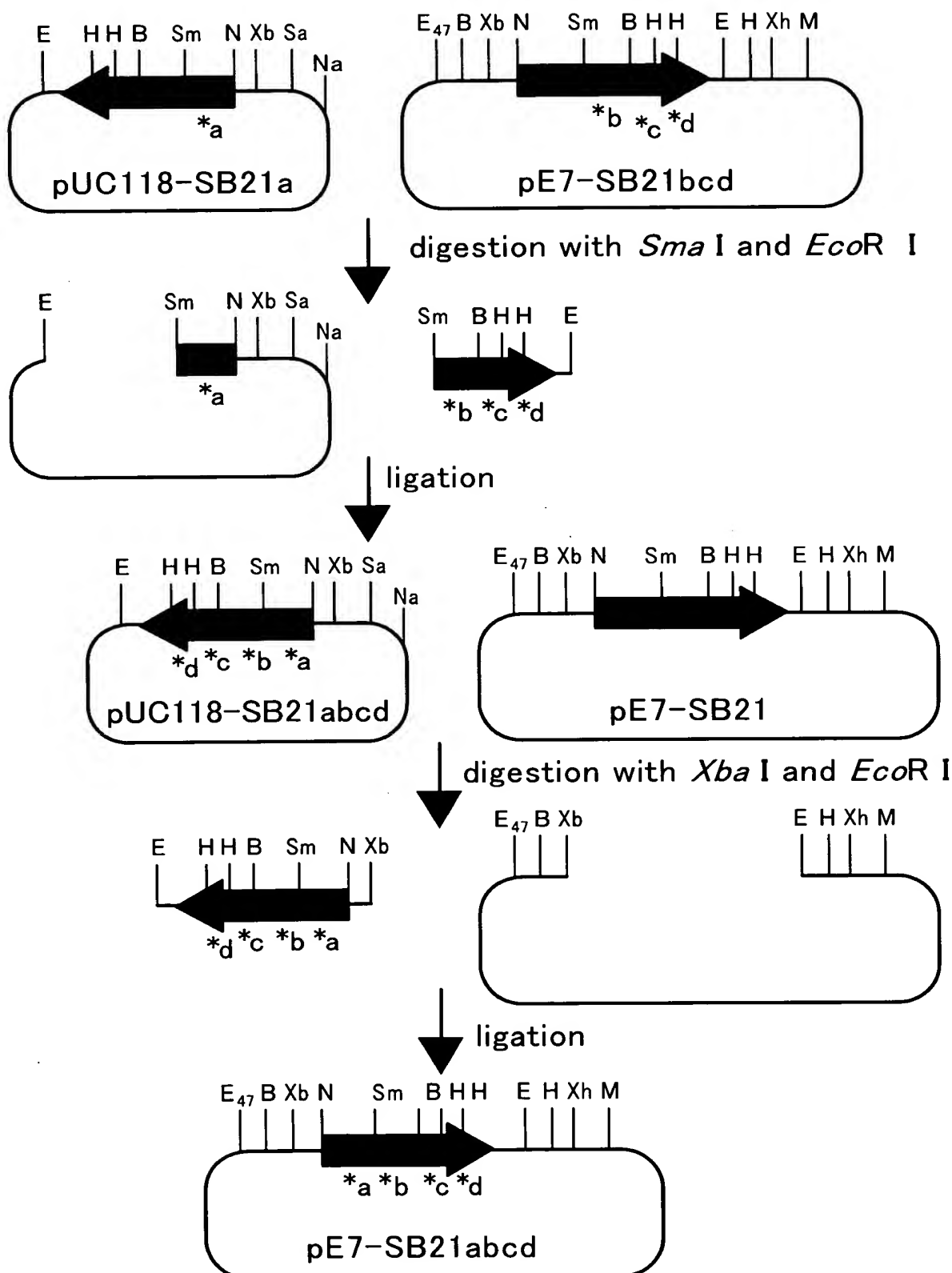
ggt aat gcg aca act gat gac tta ata aat gaa att tat aat aag cta 1008
Gly Asn Ala Thr Thr Asp Asp Leu Ile Asn Glu Ile Tyr Asn Lys Leu 336

ggc taa 1014
Gly

FIG. 7

8/13

FIG. 8



N; *Nde* I, S, m; *Sma* I, E; *Eco*R I, E₄₇; *Eco*47 III, B; *Bgl* II, X, b; *Xba* I, H; *Hind* III, X, h; *Xho* I, M; *Mro* I, Na; *Nae* I, S, a; *Sal* II,

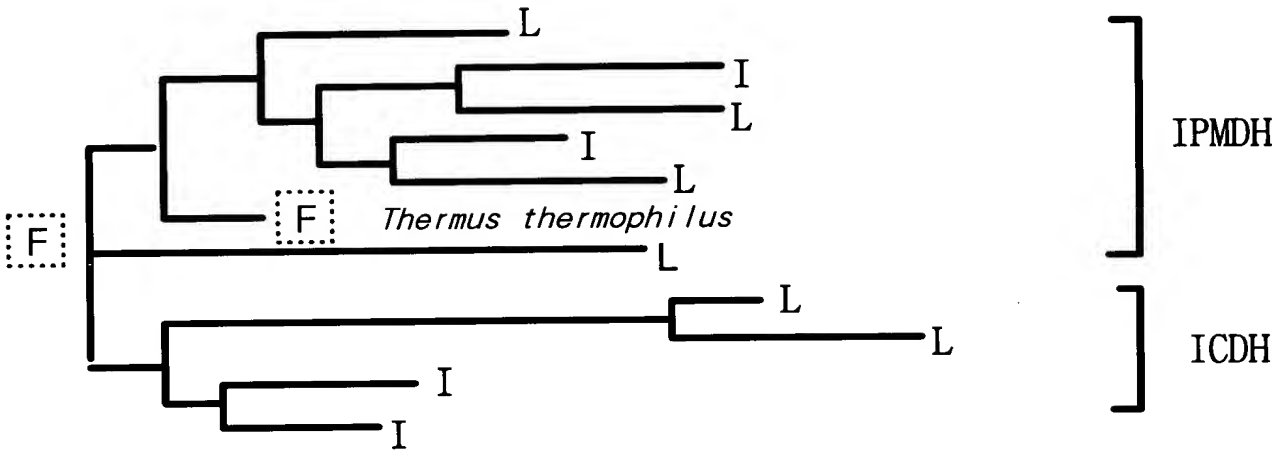
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DOCKET #: 210383US0
INV: Akihiko YAMAGISHI
SHEET 10 OF 13

10/13

FIG. 10



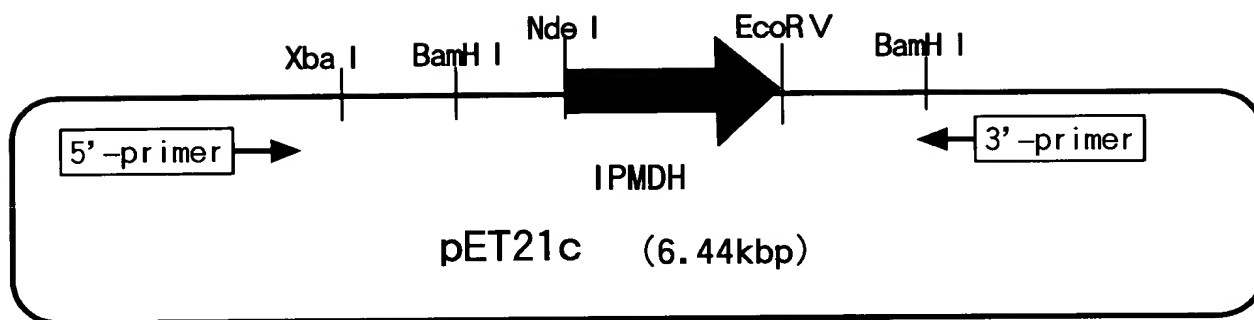
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SHEET 11 OF 13

11/13

FIG. 11



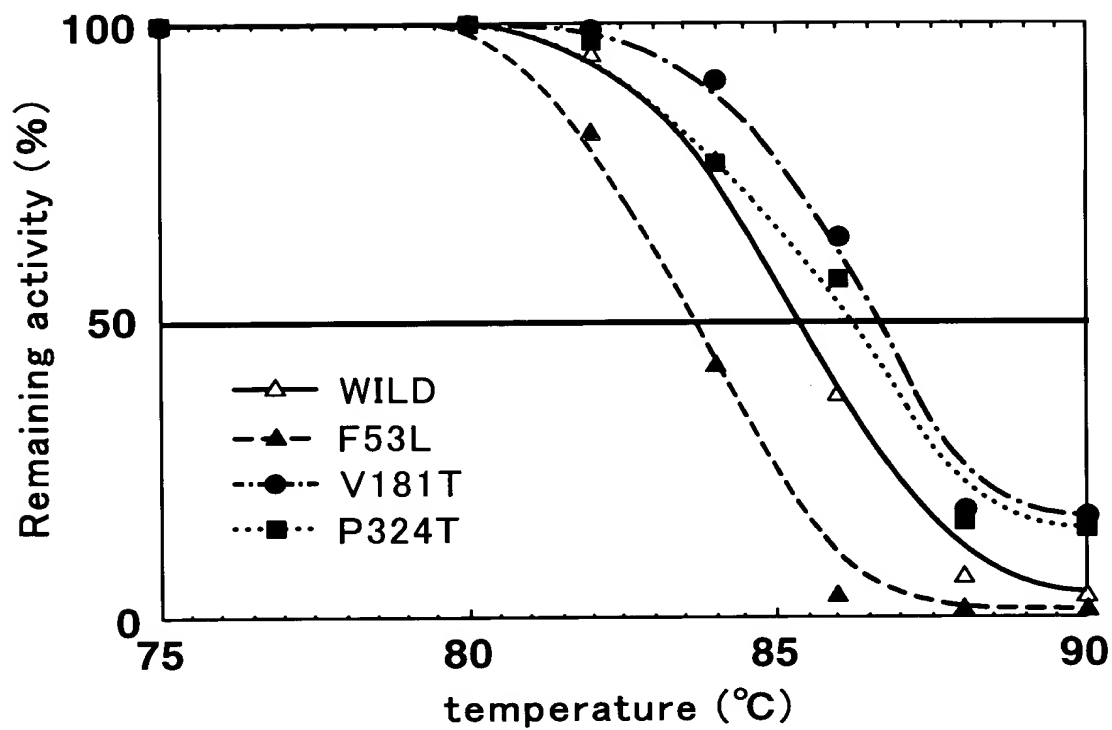
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INV: Akihiko YAMAGISHI
SHEET 12 OF 13

12/13

FIG. 12



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